

CLAIMS

1. A monoclonal antibody that specifically binds to a human VEGF with dissociation constant K_d equal to or lower than 0.2 nM.

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2. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is equal to or lower than 0.1 nM.

3. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is equal to or lower than 0.08 nM.

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4. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is equal to or lower than 0.05 nM.

5. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is equal to or lower than 0.01 nM.

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6. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is equal to or lower than 0.005 nM.

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7. The monoclonal antibody of claim 1, wherein the antibody is in a form of scFv.

8. The monoclonal antibody of claim 1, wherein the antibody is in a form of Fab.

9. The monoclonal antibody of claim 1, wherein the antibody is in a form of fully assembled antibody.

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10. The monoclonal antibody of claim 1, wherein the antibody is in a form of scFv and the dissociation constant K_d is measured at about 4°C, 25°C, 37°C or 42°C.

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11. The monoclonal antibody of claim 1, wherein the antibody is in a form of Fab and the dissociation constant K_d is measured at about 4°C, 25°C, 37°C or 42°C.

12. The monoclonal antibody of claim 1, wherein the antibody is in a form of Fab and the dissociation constant K_d is measured at about 37°C.

14. A monoclonal antibody that specifically binds to a human VEGF and has V_L comprising the amino acid sequence of

$X_1X_2X_3X_4TQX_5PSX_6X_7SX_8X_9X_{10}GX_{11}X_{12}X_{13}X_{14}IX_{15}CX_{16}X_{17}SX_{18}X_{19}IX_{20}X_{21}X_{22}X_{23}X_{24}$
10 $WYQQX_{25}PGX_{26}APX_{27}X_{28}LX_{29}YX_{30}X_{31}X_{32}X_{33}LX_{34}X_{35}GVX_{36}X_{37}RFSGX_{38}X_{39}SGTDF$
 $X_{40}LTIX_{41}X_{42}LQX_{43}X_{44}DX_{45}AX_{46}YYCQX_{47}X_{48}X_{49}X_{50}PX_{51}TFGX_{52}GTKX_{53}X_{54}IK$,
wherein the underlined regions are designated as $V_L/CDR1$, $V_L/CDR2$, and $V_L/CDR3$,
respectively, whereas the rest of the region is designated as framework, and wherein X_1 is
D, E or A; X_2 is I, or T; X_3 is V, E, K, R, Q, or T; X_4 is M, or L; X_5 is S, or T, X_6 is S,
15 or T; X_7 is L, or V; X_8 is A, or V; X_9 is S, or T; X_{10} is P, V, L, A, or I; X_{11} is E, or D; X_{12}
is R, or T; X_{13} is A, or V I; X_{14} is T, or A; X_{15} is T, S, or A; X_{16} is S, R, N, K, H, or Q;
 X_{17} is A, or S; X_{18} is Q, or R; X_{19} is S, D, A, or P; X_{20} is S, G, R, T, or Y; X_{21} is T, N, S,
D, or K; X_{22} is Y, or D; X_{23} is L, or I; X_{24} is A, N, or T; X_{25} is K, or I; X_{26} is Q, K, T, or
I; X_{27} is R, K, Q, N, H, S, or E; X_{28} is V, or L; X_{29} is I, or V; X_{30} is F, A, G, D, or S;
20 X_{31} is A, or T; X_{32} is S, or T; X_{33} is N, S, R, or T; X_{34} is A, H, or Q; X_{35} is S, or G; X_{36}
is P, T; X_{37} is S, N, D, G, or Y; X_{38} is S, or T; X_{39} is G, or R; X_{40} is T, or A; X_{41} is S, or
R; X_{42} is S, or R; X_{43} is P, or A; X_{44} is E, or D; X_{45} is F, V, or S; X_{46} is V, T, I, A, or S;
 X_{47} is Y, or S; X_{48} is S, Y, or N; X_{49} is S, or T; X_{50} is T, V, A, P, K, G, S, or I; X_{51} is W,
or Y; X_{52} is Q, or G; X_{53} is V, or L; and X_{54} is E, D, or A.

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15. A monoclonal antibody that specifically binds to a human VEGF and has V_L comprising the amino acid sequence of

$X_1X_2X_3LTQPPSX_4SX_5TPGQX_6VTISCSGX_7X_8SNX_9GX_{10}NX_{11}VX_{12}WYQQX_{13}PGX_{14}A$
 $PKX_{15}LX_{16}YX_{17}NX_{18}X_{19}RPSGVPX_{20}RX_{21}SGSX_{22}SX_{23}TSASLAISGLX_{24}SEDEADYY$
30 $CX_{25}X_{26}WDDSLX_{27}GYVFGX_{28}GTX_{29}LTVL$, wherein the underlined regions are
designated as $V_L/CDR1$, $V_L/CDR2$, and $V_L/CDR3$, respectively, whereas the rest of the

region is designated as framework, and wherein X₁ is Q L, or N; X₂ is P A F, or S; X₃ is V, or M; X₄ is A, or T; X₅ is G, or A; X₆ is R, or S; X₇ is S, or T; X₈ is S, T Y, or N; X₉ is I, or V; X₁₀ is S, or R; X₁₁ is S, P, N, A, or T; X₁₂ is N, T, or Y; X₁₃ is L, or F; X₁₄ is T, or A; X₁₅ is V, L, or F; X₁₆ is M, or I; X₁₇ is G, T, or S; X₁₈ is N, or D; X₁₉ is Q, or E; X₂₀ is D, or E; X₂₁ is F, or L; X₂₂ is K, or R; X₂₃ is G, or A; X₂₄ is Q, L, or R; X₂₅ is A, or G; X₂₆ is A, S, or T; X₂₇ is N, S, or T; X₂₈ is T, or A; and X₂₉ is K, or Q.

16. A monoclonal antibody that specifically binds to a human VEGF and has V_L comprising the amino acid sequence of
10 QSALTQPPSVSGAPGQRVTISCTGRSSNIGAGHDVHWYQQLPGTAPKLLIYANDQ
RPSGVPDRFSDSKSGTSA^{SL}GLRSEDEADYFCATWDDSLHGYVFGTGTKVTV
L (SEQ ID No: 54).

17. A monoclonal antibody is provided that specifically binds to a human VEGF and
15 has V_H comprising the amino acid sequence of
X₁X₂QLVX₃SGGGX₄VQPGGX₅LRLX₆CAX₇SGX₈X₉X₁₀X₁₁X₁₂X₁₃GX₁₄NWX₁₅RQAP
GKGX₁₆EWVGWX₁₇NTX₁₈X₁₉GX₂₀X₂₁TYX₂₂X₂₃X₂₄FX₂₅RRX₂₆TX₂₇SX₂₈X₂₉X₃₀SKX₃₁
X₃₂X₃₃YLQX₃₄NSLRAEDTAVYYCAX₃₅YPX₃₆YYGX₃₇SHWYFDVWX₃₈QGT^LLVTV
SS, wherein the underlined regions are designated as CDR1, CDR2, and CDR3,
20 respectively, whereas the rest of the region is designated as framework according to
Kabat nomenclature, and wherein X₁ is E, or Q; X₂ is V, or G; X₃ is Q, or E; X₄ is V, or
L; X₅ is S, or T; X₆ is S T, or R; X₇ is A, or V; X₈ is Y, or F; X₉ is T, D, N, S, or A; X₁₀ is
F, or L; X₁₁ is T, D, Y, A, S, or N; X₁₂ is N, H, or S; X₁₃ is Y, or F; X₁₄ is M, L, I, or V;
X₁₅ is I, V, or L; X₁₆ is L, or P; X₁₇ is I, or V; X₁₈ is Y, or N; X₁₉ is T, or N; X₂₀ is E, or
25 A; X₂₁ is P, T, or S; X₂₂ is A, or V; X₂₃ is A, H, Q, P, D, or E; X₂₄ is D, or E; X₂₅ is K, or
T; X₂₆ is V, F, or L; X₂₇ is F, or I; X₂₈ is L, or R; X₂₉ is D, or N; X₃₀ is T, or N; X₃₁ is S,
or N; X₃₂ is T, Q, P, or K; X₃₃ is A, V, or P; X₃₄ is L, or M; X₃₅ is K, or R; X₃₆ is H, or Y;
X₃₇ is S, R, or T; and X₃₈ is G, or A.

30 18. A monoclonal antibody is provided that specifically binds to a human VEGF and
has V_L comprising the amino acid sequence selected from the group consisting of SEQ ID

NOs:2-54, more preferably comprising the amino acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:47, and SEQ ID NO:54.

- 5 19. A monoclonal antibody that specifically binds to a human VEGF and has V_H comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:57-110 and SEQ ID NOs:285-310, and preferably comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:61-64, SEQ ID NO:67, 68, 70, 75, 83, 88, 89, 90, 91, 92, 93, 94, and 96-110.

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20. A monoclonal antibody is provided that specifically binds to a human VEGF and has CDR2 in the V_L region (V_L/CDR2) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:195-209.

- 15 21. A monoclonal antibody that specifically binds to a human VEGF and has CDR3 in the V_L region (V_L/CDR3) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:210-228.

22. A monoclonal antibody that specifically binds to a human VEGF and has a
20 framework region (FR) CDR3 in the V_L region (V_L/FR) comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:229-269, and preferably comprising the amino acid sequence selected from the group consisting of SEQ ID NO:232, 235, 237, 251, 255, 263, and 265.

- 25 23. A monoclonal antibody that specifically binds to a human VEGF and has CDR1 in the V_H region (V_H/CDR1) comprising the amino acid sequence of GX₁X₂X₃X₄X₅X₆GX₇N, wherein X₁ is Y, or F; X₂ is D, N, T, S, or A; X₃ is F, or L; X₄ is T, D, S, Y, A, or N; X₅ is H, N, or S; X₆ is Y, or F; X₇ is M, L, I, or V.

- 30 24. A monoclonal antibody that specifically binds to a human VEGF and has CDR2 in the V_H region (V_H/CDR2) comprising the amino acid sequence of

WX₁NTX₂X₃GEX₄TYX₅X₆X₇FX₈R, wherein X₁ is I, or V; X₂ is Y, or N; X₃ is T, or N; X₄ is P, T, or S; X₅ is A, or V; X₆ is A, Q, P, H, D, or E; X₇ is D, or E; and X₈ is K, or T.

25. A monoclonal antibody that specifically binds to a human VEGF and has CDR2
5 in the V_H region (V_H/CDR2) comprising the amino acid sequence selected from the group consisting of: SEQ ID NOs:136-156.

26. A monoclonal antibody that specifically binds to a human VEGF and has CDR3
10 in the V_H region (V_H/CDR3) comprising the amino acid sequence of KYPX₁YYGX₂SHWYFDV, wherein X₁ is Y, or H, and X₂ is R.

27. A monoclonal antibody that specifically binds to a human VEGF and has CDR3
15 in the V_H region (V_H/CDR3) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:311-337.

28. A monoclonal antibody that specifically binds to a human VEGF and has FR in
the V_H region (V_H/FR) comprising the amino acid sequence of
X₁VQLVX₂SGGGX₃VQPGGX₄LRLX₅CAX₆S/CDR1/WX₇RQAPGKGGLEWVG/CDR2/
RX₈TX₉SX₁₀DX₁₁SKX₁₂X₁₃X₁₄YLQX₁₅NSLRAEDTAVYYCA/CDR3/WX₁₆QGTLVTV
20 SS, wherein X₁ is E, or Q; X₂ is Q, or E; X₃ is V, or L; X₄ is S, or T; X₅ is S, T, or R; X₆
is A, or V; X₇ is I, or V; X₈ is F, or V; X₉ is F, or I; X₁₀ is L, or R; X₁₁ is T, or N; X₁₂ is
S, or N; X₁₃ is T, Q, or K; X₁₄ is A, or V; X₁₅ is M, or L; and X₁₆ is G, or A.

29. A monoclonal antibody that specifically binds to a human VEGF and has a V_L
25 and V_H pair selected from the group consisting of: SEQ ID NO:1 and 70; SEQ ID NO:1
and 67; SEQ ID NO:1 and 75; SEQ ID NO:1 and 83; SEQ ID NO:14 and 55; SEQ ID
NO:1 and 101; SEQ ID NO:1 and 100; SEQ ID NO:14 and 102; SEQ ID NO:1 and 103;
SEQ ID NO:1 and 104; SEQ ID NO:1 and 105; SEQ ID NO:36 and 100; SEQ ID NO:26
and 100; SEQ ID NO:28 and 100; SEQ ID NO:37 and 100; SEQ ID NO:44 and 100;
30 SEQ ID NO:54 and 100; and SEQ ID NO:47 and 100, preferably selected from the group
consisting of SEQ ID NO:28 and 61; SEQ ID NO:28 and 62; SEQ ID NO:28 and 63;

SEQ ID NO:28 and 64; SEQ ID NO:28 and 68; SEQ ID NO:28 and 85; SEQ ID NO:28
and 86; SEQ ID NO:28 and 87; SEQ ID NO:28 and 88; SEQ ID NO:28 and 89; SEQ ID
NO:28 and 90; SEQ ID NO:28 and 91; SEQ ID NO:28 and 92; SEQ ID NO:28 and 93;
SEQ ID NO:28 and 94; SEQ ID NO:28 and 95; SEQ ID NO:28 and 96; SEQ ID NO:28
5 and 97; SEQ ID NO:28 and 98; SEQ ID NO:28 and 99; SEQ ID NO:28 and 106; SEQ ID
NO:28 and 107; SEQ ID NO:28 and 108; SEQ ID NO:28 and 109; and SEQ ID NO:28
and 110.

30. The monoclonal antibody of any of claims 14-29, wherein the antibody has
10 dissociation constant K_d equal to or lower than 10 nM.

31. The monoclonal antibody of any of claims 14-29, wherein the antibody has
dissociation constant K_d equal to or lower than 1 nM.

15 32. The monoclonal antibody of any of claims 14-29, wherein the antibody has
dissociation constant K_d equal to or lower than 0.1 nM.

33. The monoclonal antibody of any of claims 14-29, wherein the antibody has
dissociation constant K_d equal to or lower than 0.01 nM.

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